IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Ning Hu et al.	Art Unit	: 1612
Serial No.	: 10/723,431	Examiner	: G.S. Kishore
Filed	: November 26, 2003	Docket	: 01992.007US1
Title	: METHOD OF DRUG LOADING IN LIPOSOMES BY GRADIENT		

REPLY BRIEF

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Applicant hereby submits this Reply Brief in response to the Examiner's Answer mailed on May 11, 2010.

If necessary, please charge any required fees or credit overpayment to Deposit Account 50-3503.

i) REAL PARTY IN INTEREST

The real party in interest is Gilead Sciences, Inc. The right of Gilead Sciences Inc. to take action in the subject application was established by virtue of an assignment from the inventors to Gilead Sciences Inc. as recorded at Reel 016496, Frame 0788.

ii) RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences.

iii) STATUS OF CLAIMS

The final Office action mailed on January 12, 2009 rejected claims 1-28, 30, 31, 33, 40-42 and 47-71. No claims have been allowed. Claims 29, 32, 34-39 and 43-46 have been canceled. Therefore, the rejection of claims 1-28, 30, 31, 33, 40-42 and 47-71 is respectfully appealed.

iv) STATUS OF AMENDMENTS

No amendments have been filed subsequent to the final Office action dated January 12, 2009.

v) SUMMARY OF CLAIMED SUBJECT MATTER

The invention provides a method for encapsulation of pharmaceutical agents in liposomes having a high drug:lipid ratio. The invention also includes gradient loaded liposomes prepared by these methods as well as methods to prepare pharmaceutical compositions comprising said liposomes.

Independent Claim 1 provides a method of forming a gradient loaded liposome. Support for claim 1 can be found at page 18, line 1, page 23, line 27 and page 13, line 24.

A method of forming gradient loaded liposomes, the method comprising:

(a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent selected from an anthracycline chemotherapeutic agent, an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;

(c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes; and

(d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes.

Independent Claim 63 provides a method for preparing a pharmaceutical composition of a gradient loaded liposome. Support for claim 63 can be found at page 26, line 1, page 23, line 27 and page 13, line 24.

A method for preparing a pharmaceutical composition comprising:

(a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent selected from an anthracycline chemotherapeutic agent,

an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;

(c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes;

(d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes; and

(e) combining the gradient loaded liposomes with a pharmaceutically acceptable carrier to provide the pharmaceutical composition.

Independent Claim 71 provides a gradient loaded liposome prepared by a process of the invention. Support for claim 71 can be found at page 26, line 17, page 23, line 27 and page 13, line 24.

A gradient loaded liposome prepared by the process comprising:

(a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent selected from an anthracycline chemotherapeutic agent, an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;

(c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes; and

(d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes.

vi) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

(1) Whether claims 1-28, 30-31, 33, 40-42 and 47-71 are unpatentable under 35 U.S.C. 103(a) over WO 99/13816 in combination with Tardi (US 2003/0124181).

(2) Whether claims 7 and 49 are unpatentable under 35 U.S.C. 103(a) over WO 99/13816 in combination with Tardi, further in view of Webb (5,814,335).

(3) Whether claims 52-57 are unpatentable under 35 U.S.C. 103(a) over WO 99/13816 in combination with Tardi (US 2003/0124181), further in view of Clerc (5,939,096).

(4) Whether claims 1-28, 30-31, 33, 40-42 and 47-71 are unpatentable on the grounds of nonstatutory obviousness-type double patenting over claims 30-31 and 35-64 of U.S. Patent No. 6,740,335 in combination with Tardi (US 2003/0124181).

vii) ARGUMENT

The Examiner bears the initial burden to establish a *prima facie* case of obviousness. It is respectfully reasserted that the Examiner has not met the burden to establish a *prima facie* case of obviousness over WO 99/13816 in combination with Tardi.

WO 99/13816 teaches the loading of liposomes with camptothecins. However, it does not teach the loading of an anthracycline chemotherapeutic agent, an anthracenedione or a vinca alkaloid as recited in instant claim 1. To correct this deficiency in WO 99/13816 the Examiner relies on the disclosure of the secondary reference Tardi, which generally discusses the loading of camptothecins, anthracycline chemotherapeutic agents, anthracenediones and vinca alkaloids into a liposome using a pH gradient.

The liposome loading methods discussed in WO 99/13816 and the liposome loading methods of the instant claims differ significantly from the loading methods discussed in Tardi. In particular, the liposome loading methods discussed in WO 99/13816 and the liposome loading methods of the instant claims include a cooling step (c) and a quenching step (d). The quenching step recited in independent claims 1, 63, and 71 includes contacting the liposome solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome. This quenching step removes the pH gradient from the final liposome.

Tardi does not teach the preparation of any liposomes using a quenching step. Accordingly, the final liposomes prepared by Tardi maintain a low pH in the internal aqueous space that helps keep the drug loaded inside the liposome. At paragraph 0076 Tardi teaches that "Once the drug moves inside the liposome, the pH of the interior results in a charged drug state, which prevents the drug from permeating the lipid bilayer, thereby entrapping the drug in the liposome." Additionally in paragraph 0080, Tardi teaches that "Conversion of moiety to a charged form causes the drug to remain encapsulated within the liposome." Accordingly, Tardi teaches that it is critical to maintain the low pH in the internal aqueous space after active loading of the liposome in order to keep the therapeutic agent trapped inside.

In light of this specific teaching of Tardi, it is respectfully submitted that that one skilled in the art would not have been motivated to combine the teachings of WO 99/13816 and Tardi as suggested by the Examiner. Such a combination would have provided loaded liposomes wherein the pH gradient had been quenched. However, Tardi specifically teaches that the pH gradient must be maintained to keep the drug from permeating the lipid bilayer in the final liposome. Additionally, even if one skilled in the art had been motivated to combine Tardi and WO 99/13816 as suggested by the Examiner, one skilled in the art would not have had a reasonable expectation that the resulting liposomes would be useful. Again, Tardi specifically teaches that the pH gradient is necessary to keep the drugs encapsulated in the final liposomes.

At page 9 of the Examiner's Answer the Examiner states that Tardi teaches "the equivalency between camptothecins and claimed anthracyclines and vinca alkaloids." Although Tardi does generally discuss loading liposomes with camptothecins, anthracyclines and vinca alkaloids, the final liposomes provided in Tardi differ significantly from the final liposomes discussed in WO 99/13816. The final liposomes of Tardi possess a pH gradient that is taught by Tardi to be essential. Due to this clear and specific teaching in Tardi, it is submitted that one skilled in the art would not have had any motivation to load the drugs generally discussed in Tardi using the methods of WO 99/13816, which include a quenching step that eliminates the pH gradient from the final liposomes.

Since there was no motivation to combine the cited documents as suggested by the Examiner, and because there would not have been a reasonable expectation that the references so combined would have provided liposomes that would have sufficiently retained the therapeutic agent, it is respectfully submitted that the Office has not established a *prima facie* case of obviousness over WO 99/13816 in combination with Tardi.

Additionally, independent claims 1, 63 and 71 include the loading of liposomes using an aqueous solution of at least about 60 mM of an acid. Claims 2-28, 30, 31, 33, 40-42, 47-62 and 64-70 are directly or indirectly dependent on claims 1, 63 and 71. The recited concentration of 60 mM of an acid is not taught by WO 99/13816. The Examiner has taken the position "although in examples, WO uses citric acid at 50 mM

concentration, in view of WO's teachings that it can be higher than 5 mM, it would have been obvious to one of ordinary skill in the art to vary the molality with the expectation of obtaining the best possible results." The Examiner has provided no evidence to support the conclusion that one skilled in the art, upon reading WO 99/13816, would have been motivated to use a higher concentration (i.e. greater than 50 mM) of acid in loading liposomes. In addition, the Examiner has provided no rebuttal to the Arguments presented in the Appeal Brief regarding the preferred buffer concentration of 50 mM described in WO 99/13816. Accordingly, it is submitted that WO 99/13816 does not teach the acid concentration recited in independent claims 1, 63 and 71.

In conclusion, one skilled in the art would not have been motivated to combine WO 99/13816 and Tardi as suggested by the Examiner. Additionally, even if the cited documents were combined, *arguendo*, the combination would still not teach the acid concentration recited in independent claims 1, 63, and 71. Thus, it is respectfully submitted that the pending claims are not obvious in view of the cited documents individually or in combination. For these reasons Applicant requests that the Board reverse the Examiner's rejection of claims 1-28, 30-31, 33, 40-42 and 47-71 over WO 99/13816 in combination with Tardi. For similar reasons Applicant also requests that the Board reverse the Examiner's rejection of claims 1-28, 30-31, 33, 40-42 and 47-71 on the ground of nonstatutory obviousness-type double patenting over U.S. Patent No. 6,740,335 (of which WO 99/13816 is a foreign counterpart) in combination with Tardi.

Applicant respectfully submits that the claims are in condition for allowance, and notification to that effect is respectfully requested. If necessary, please charge any additional fees or credit overpayment to Deposit Account 50-3503.

Respectfully submitted,

Date: July 12, 2010 By: _____

John Mickelson (Reg. No. 63,638)

Viksnins Harris & Padys PLLP
Customer Number 53137
PO Box 111098
St. Paul, MN 55111-1098
(952) 876-4089

CERTIFICATE OF TRANSMISSION
I hereby certify that this correspondence is transmitted electronically by facsimile, by electronic transmission, or is being deposited with the United States Postal Service as first class mail with sufficient postage to the Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450 this 12 day of July 2010.

Michelle McCullough
Typed or Printed Name of Person Signing Certificate

Signature

viii) CLAIMS APPENDIX

1. A method of forming gradient loaded liposomes, the method comprising:

(a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent selected from an anthracycline chemotherapeutic agent, an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;

(c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes; and

(d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes.

2. The method of claim 1 wherein the liposomes comprise phosphatidylcholine.

3. The method of claim 1 wherein the liposomes comprise phosphatidylcholine selected from the group of distearoylphosphatidylcholine, hydrogenated soy phosphatidylcholine, hydrogenated egg phosphatidylcholine, dipalmitoylphosphatidylcholine, dimyristoylphosphatidylcholine, and dielaidoyl phosphatidyl choline.

4. The method of claim 1 wherein the liposomes further comprise cholesterol.

5. The method of claim 1 wherein the liposomes further comprise phosphatidylglycerol.

6. The method of claim 1 wherein the liposomes further comprise non-phosphatidyl lipids.

7. The method of claim 6 wherein the non-phosphatidyl lipids comprise sphingomyelin.

8. The method of claim 1 wherein the liposomes further comprise phosphatidylglycerol selected from the group of dimyristoylphosphatidylglycerol, dilaurylphosphatidylglycerol, dipalmitoylphosphatidylglycerol, and distearoylphosphatidylglycerol.

9. The method of claim 1 wherein the liposomes comprises phosphatidylcholine, and further comprises cholesterol.

10. The method of claim 1 wherein the liposomes comprises phosphatidylcholine, and further comprises cholesterol, wherein the molar ratio of the phosphatidylcholine to the cholesterol is about 1:0.01 to about 1:1.

11. The method of claim 1 wherein the liposomes comprises phosphatidylcholine, and further comprises cholesterol, wherein the molar ratio of the phosphatidylcholine to the cholesterol is about 1.5:1.0 to about 3.0:1.0.

12. The method of claim 1 wherein the liposomes are unilamellar and less than about 100nm.

13. The method of claim 1 wherein the weight ratio of the liposomes to the pharmaceutical agent is up to about 200:1.

14. The method of claim 1 wherein the weight ratio of the liposomes to the pharmaceutical agent is about 1:1 to about 100:1.

15. The method of claim 1 wherein the weight ratio of the liposomes to the pharmaceutical agent is about 1:1 to about 50:1.

16. The method of claim 1 wherein the acid has an acid dissociation constant of less than about 1×10^{-2}.

17. The method of claim 1 wherein the acid has an acid dissociation constant of less than about 1×10^{-4}.

18. The method of claim 1 wherein the acid has an acid dissociation constant of less than about 1×10^{-5}.

19. The method of claim 1 wherein the acid has a permeability coefficient larger than about 1×10^{-4} cm/sec for the liposomes.

20. The method of claim 1 wherein the acid is selected from the group of formic acid, acetic acid, propanoic acid, butanoic acid, pentanoic acid, citric acid, oxalic acid, succinic acid, lactic acid, malic acid, tartaric acid, fumaric acid, benzoic acid, aconitic acid, veratric acid, phosphoric acid, sulfuric acid, and combinations thereof.

21. The method of claim 1 wherein the acid is citric acid.

22. The method in claim 1 wherein at least about 100 mM of an acid is employed.

23. The method of claim 1 wherein the pharmaceutical agent exists in a charged state when dissolved in an aqueous medium.

24. The method of claim 1 wherein the pharmaceutical agent is an organic compound that includes at least one acyclic or cyclic amino group, capable of being protonated.

25. The method of claim 1 wherein the pharmaceutical agent is an organic compound that includes at least one primary amine group, at least one secondary amine group, at

least one tertiary amine group, at least one quaternary amine group, or any combination thereof.

26. The method of claim 1 wherein the pharmaceutical agent is an antineoplastic agent.

27. The method of claim 1 wherein the pharmaceutical agent is a combination of two or more antineoplastic agents.

28. The method of claim 1 wherein the pharmaceutical agent is an ionizable basic antineoplastic agent.

30. The method of claim 1 wherein the anthracycline chemotherapeutic agent is selected from the group of doxorubicin, epirubicin, and daunorubicin.

31. The method of claim 1 wherein the anthracenedione is mitoxantrone.

33. The method of claim 1 wherein the vinca alkaloid is selected from the group of vincristine and vinblastine.

40. The method of claim 1 wherein the temperature in step (a) is about 40°C to about 70°C.

41. The method of claim 1 wherein the temperature in step (a) is about 50°C to about 60°C.

42. The method of claim 1 wherein the solution is cooled in step (c) to a temperature of about 0°C to about 30°C.

47. The method of claim 1 wherein the weak base is an ammonium salt having a mono- or multi-valent counterion.

48. The method of claim 1 wherein the weak base is selected from the group of ammonium sulfate, ammonium hydroxide, ammonium acetate, ammonium chloride, ammonium phosphate, ammonium citrate, ammonium succinate, ammonium lactobionate, ammonium carbonate, ammonium tartarate, ammonium oxalate, and combinations thereof.

49. The method of claim 1 wherein the weak base is alkyl-amine selected from the group of methyl amine, ethyl amine, diethyl amine, ethylene diamine, and propyl amine.

50. The method of claim 1 further comprising, during or after step (d), removing any unloaded pharmaceutical agent.

51. The method of claim 50 wherein the removing of the unloaded drug employs removing the unloaded drug via cross filtration or dialysis.

52. The method of claim 1 further comprising, after step (d), dehydrating the liposomes.

53. The method of claim 52 wherein the dehydrating is carried out at a pressure of below about 1 atm.

54. The method of claim 52 wherein the dehydrating is carried out with prior freezing of the liposomes.

55. The method of claim 52 wherein the dehydrating is carried out in the presence of one or more protective monosaccharide sugars, one or more protective disaccharide sugars, or a combination thereof.

56. The method of claim 55 wherein the protective sugar is selected from the group of trehalose, sucrose, maltose, and lactose.

57. The method of claim 52 further comprising rehydrating the liposomes after the dehydrating.

58. The method of claim 1 wherein the liposomes are unilamellar vescicles.

59. The method of claim 1 wherein the liposomes are multilamellar vescicles.

60. The method of claim 1 wherein more than about 90 wt.% of the pharmaceutical agent is trapped in the liposomes.

61. The method of claim 1 further comprising, after step (d), contacting the liposomes with a pharmaceutically acceptable carrier.

62. The method of claim 1 wherein the acid is present in at least about 200 mM.

63. A method for preparing a pharmaceutical composition comprising:

(a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent selected from an anthracycline chemotherapeutic agent, an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;

(c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes;

(d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes; and

(e) combining the gradient loaded liposomes with a pharmaceutically acceptable carrier to provide the pharmaceutical composition.

64. A method comprising administering the pharmaceutical composition of claim 63 to a mammal.

65. A method for treating a mammal inflicted with cancer, the method comprising administering the pharmaceutical composition of claim 63 to the mammal, wherein the pharmaceutical agent is an antineoplastic agent.

66. The method of claim 65 wherein the cancer is a tumor, ovarian cancer, small cell lung cancer (SCLC), non small cell lung cancer (NSCLC), leukemia, sarcoma, colorectal cancer, head cancer, neck cancer, or breast cancer.

67. The method of claim 65 wherein the administration of the antineoplastic agent, *via* the liposomal formulation, has a toxicity profile that is lower than the toxicity profile associated with the administration of the antineoplastic agent in the free form.

68. The method of claim 67 wherein the toxicity is selected from the group of gastrointestinal toxicity and cumulative dose-dependent irreversible cardiomyopathy.

69. The method of claim 65 wherein the administration of the antineoplastic agent has unpleasant side-effects that are lower in incidence, severity, or a combination thereof,

than unpleasant side-effects associated with the administration of the antineoplastic agent in the free form.

70. The method of claim 69 wherein the unpleasant side-effects are selected from the group of myelosuppression, alopecia, mucositis, nausea, vomiting, and anorexia.

71. A gradient loaded liposome prepared by the process comprising:

(a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent selected from an anthracycline chemotherapeutic agent, an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;

(c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes; and

(d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes.

ix) EVIDENCE APPENDIX

A. WO 99/13816

This document was submitted in an Information Disclosure Statement mailed February 9, 2005.

B. US 2003/0124181

This document was entered by the Examiner in the Office Action dated May 14, 2008.

C. US 5,814,335

This document was submitted in an Information Disclosure Statement mailed February 9, 2005.

D. US 5,939,096

This document was entered by the Examiner in the Office Action dated March 27, 2007.

x) RELATED PROCEEDINGS APPENDIX

None.